THE MAJOR PROTEINS OF THE *ESCHERICHIA COLI* OUTER CELL ENVELOPE MEMBRANE: EVIDENCE FOR THE STRUCTURAL GENE OF PROTEIN II*

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1. Introduction

The outer cell envelope membrane of *E. coli* contains a set of major (major concerning their cellular concentrations) proteins in a mol. wt. range between 10 000 and 38 000; the present knowledge on these proteins has recently been briefly summarized [1]. One of these proteins we have named protein II*. We have reported that selection for resistance to a certain phage (phage TuII*) can yield mutants that no longer possess protein II* [2] or produce this protein in an altered form [1], and we have now established that the phage uses protein II* as a receptor (I. Sonntag and U. Henning, in preparation). Here we describe the chromosomal location of such phage resistant mutations and we provide evidence that this locus, provisionally called *tut*, appears to represent the structural gene for protein II*.

2. Experimental

Strain W620 (3, *thi*, *pyr*D, *glt*A) was from Dr H. U. Schairer, and strain YAA1 (4, *thi*, *his*, *trp*, *fab*A) from Dr B. Bachmann from the Coli Genetic Stock Center at Yale University. The TuII* resistant mutants P692 *tut*2eI and P530 *tut*1cII have been described [1]. Transduotion with phage P1 was performed according to Lennox [5]. Preparation of cell envelopes and conditions of SDS-polyacrylamide gel electrophoresis were done as described repeatedly, e.g. [2].

3. Results

3.1. *Chromosomal localization of the* tut *locus*

Two different loci have been described where mutation can lead to absence of protein II* from the outer membrane. Foulds [6] has shown that mutations conferring tolerance to a certain colicin (*tol*G locus) map between the *pyr*D and *fab*A loci. Chai and Foulds [7] demonstrated that such mutants miss a major outer membrane protein and this protein was found to be identical with our protein II* [2]. Davies and Reeves showed that a *con* locus is cotransducible with *lip* [8]. Skurray et al. [9] had found that such mutants, deficient as recipients in conjugation, can also miss a major outer membrane protein that most likely is also identical with our protein II*. *Con* and *pyr*D are about 7 minutes apart on the *E. coli* chromosome.

Cotransduction of the TuII*^{R} character with *lip* and *pyr*D was tested with 15 different *tut* mutants and all of them were linked (~ 60%) to *pyr*D. The data of table 1 agree well with the order *pyr*D - *tut* - *fab*A (fig.1). It thus is likely that *tol*G and *tut* are identical loci.

3.2. *Evidence for* tut *being the structural gene for protein II**

As mentioned above, selection for resistance to phage TuII* yields two classes of mutants, one missing and one still possessing protein II* in their outer membrane. Among those still incorporating protein II* into the outer membrane two mutants

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Table 1
Three factor crosses of *tut* with *fab*A and *pyr*D

Strains, relevant markers		Selected marker	Segregation of unselected markers (number of selected recombinants tested)
Donor	Recipient		
W620 *pyr*D, *tut*	YAA1 *fab*A	*fab*A$^+$	*pyr*D$^+$*tut*$^+$ (31) *pyr*D$^+$*tut* (89) *pyr*D*tut*$^+$ (6) *pyr*D*tut* (120)
YAA1 *fab*A	W620 *pyr*D, *tut*	*pyr*D$^+$	*fab*A$^+$*tut*$^+$ (8) *fab*A$^+$*tut* (78) *fab*A*tut*$^+$ (66) *fab*A*tut* (1)

The genetic nomenclature is that of Taylor and Trotter [11]. The *tut* allele in W620 was isolated as a spontaneous mutant resistant to phage TuII* that still possesses protein II*.

were found where protein II* exhibits an altered electrophoretic mobility [1]. These two mutants were used as donors in phage P1 mediated transduction, selecting for *pyr*$^+$ in a phage TuII* sensitive, *pyr*D recipient (W620). *Pyr*$^+$ *tut* recombinants were isolated and it was found that their proteins II* had the same altered electrophoretic mobility as the respective donors (fig.2). The two altered proteins were isolated [10] and subjected to cyanogen bromide cleavage. The result is shown in fig.2. Wild type protein II* yields the cyanogen bromide fragments *a, b, c, d,* and *e.* In both mutant proteins fragments *b* and *c* are missing. The cyanogen bromide fragments of protein II* have not yet been isolated but preliminary experiments indicate that *b* and *c* may be overlapping fragments, i.e., a large part of *b* may be identical with *c.* If so, the simplest interpretation would be that in one mutant protein an amino acid residue X has been replaced by methionine. A new, smaller fragment can be seen in this case (fig.2,6) and the second new fragment to be expected may be too small as to be detectable. A straight forward explanation is not yet possible for the fragments of the other mutant protein which migrates somewhat slower in electrophoresis than the wild type species.

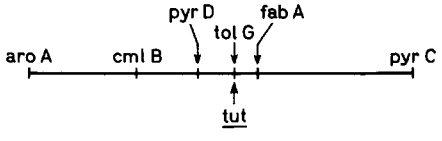

Fig.1. Position of *tut* on the *E. coli* chromosome. This chromosomal section corresponds to about 20.5 to 23.5 min. on the *coli* linkage map ([11] cf. also [6]).

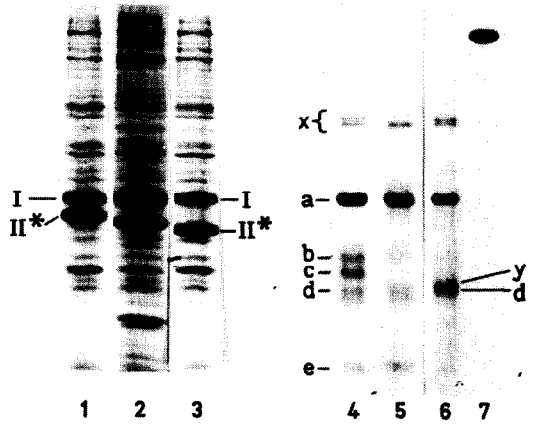

Fig.2. SDS-electrophoreses of mutant proteins II* and their cyanogen bromide fragments. 1–3, cell envelopes. 1, *pyr*$^+$*tut* recombinant from transduction P530 *tut*1cII (donor) into W620; 2, wild type (W620 *tut*$^+$); 3, *pyr*$^+$*tut* recombinant from transduction P692 *tut*2eI into W620. The same altered mobilities of the two mutant proteins from their parents have been demonstrated before [1]. Protein I is another major outer cell envelope membrane protein (see e.g., 2). 4–6, cyanogen bromide fragments. 4, wild type protein II*; 5, protein from P530 *tut*1cII; 6, protein from P692 *tut*2eI. 7, protein II*. X, incompletely cleaved fragments. The new fragment Y in 6 is hardly separable from d and d stains with an almost red color, therefore, the presence of the two bands Y and d is clearly visible only by inspection of the gels. A number of weakly staining bands (e.g., above b and below d in 4, between a and d in 5) very probably do not represent main cleavage products.

4. Discussion

The data presented show that mutations at the *tut* locus (that very likely is identical with Foulds' *tol*G locus) can cause the production of altered proteins II*. The chemical difference between altered and wild type proteins is not yet known but it can be traced to a pair of cyanogen bromide fragments and, therefore, should definitely reflect a change in the primary structure of the protein (protein II* has very little, if any, non-protein substituents [1]).

It cannot be excluded that protein II* undergoes a post-translational modification (e.g., removal of a terminal peptide) before incorporation into the outer membrane. Therefore, a change in the primary structure of the protein may be due to mutation regarding a modification system and not to protein II* structural gene mutation. Such a possibility, however, is fairly unlikely. Of 60 *tut* isolates 8 have been found that still possess protein II* and 6 of these do not show an altered electrophoretic mobility. All 8 map at the *tut* locus. As mentioned above, protein II* is a receptor for phage TuII*; 3 of the latter mutants (including one with an electrophoretically altered protein) have been tested for their ability to adsorb the phage and they failed to do so. It appears rather difficult to visualize a modification of the protein's primary structure which would be consistent with these facts *and* the assumption that *tut* mutants concern the hypothetical modification system and not the structural gene in question. We conclude that in high probability *tut* represents the structural gene for protein II*.

Preliminary experiments have shown that *tut* mutants missing protein II* are deficient as recipients in conjugation (similar to the *con* mutants mentioned above), while a *tut* mutant still possessing protein II* did not exhibit such a deficiency. The possibility exists that both phage and donor in conjugation use protein II* as a receptor but recognize different sites of the protein at the cell surface.

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